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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Taka-Aki Sato Examiner: J. Kerr

Serial No.: 09/327,750 Art Unit: 1633

Filed : June 7, 1999

For : GENE ENCODING NADE, P75^{NTR}-ASSOCIATED CELL DEATH EXECUTOR AND USES THEREOF

1185 Avenue of the Americas
New York, New York 10036
June 15, 2001

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

AMENDMENT IN RESPONSE TO JUNE 1, 2001 COMMUNICATION REGARDING NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

This Amendment is submitted in response to the June 1, 2001 Communication Regarding Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures issued by the United States Patent and Trademark Office in connection with the above-identified application. A copy of the Notice to Comply is attached hereto as **Exhibit A.** A response to the June 1, 2001 Notice is due July 1, 2001. Accordingly, this Amendment is being timely filed.

Please amend the subject application as follows:

In the Specification:

Please amend the paragraphs of the specification identified below:

Please amend the paragraph on page 11, lines 3-10. A clean version of the amended paragraph follows:

Figure 1A

Amino acid alignment of mouse (SEQ. ID NO:12) and human NADE (HGR74) (4) proteins (SEQ. ID NO:13). The dotted sequence is asparagine rich stretch. The asterisks indicate the leucine-rich nuclear export signal (NES)(5). The closed triangle indicates cysteine residue essential for dimmer formation. The prenylation sequence in C-termini is underlined.

Please amend the paragraph on page 11, line 12-19. A clean version of the amended paragraph follows:

Figure 1B

Comparison of leucine-rich nuclear export signal (NES) (5) in various protein. The consensus sequence for NES are shadowed. Genbank accession numbers are: cZyxin, X69190 (SEQ. ID NO:14); MAPKK, D13700 (SEQ. ID NO:15); PKI-a, L02615 (SEQ. ID NO:16); TFIIIA, M85211 (SEQ. ID NO:17); RevHIV-1, AF075719 (SEQ. ID NO:18); RanBP1, L25255 (SEQ. ID NO:19); FMRP, L29074 (SEQ. ID NO:20); Gle1, U68475 (SEQ. ID NO:21); RexHTLV-1 ((SEQ. ID NO:22); Human NADE (SEQ. ID NO:23), submitted; mouse NADE (SEQ. ID NO:24), submitted.

Please amend the paragraph on page 11, lines 21-22. A clean version of the amended paragraph follows:

Figure 1C

Consensus sequence of ubiquitination signal, Mouse (SEQ. ID NO:25); Human (SEQ. ID NO:26) and Consensus (SEQ.ID NO:27).

Please amend the paragraph on page 12, lines 10-13. A clean version of the amended paragraph follows:

Figure 1G-1 and 1G-2

Blast Search and comparison of mouse NADE nucleic acid sequence

Figure 1G-1 (SEQ ID NO:28) and human protein HGR74 sequence (SEQ. ID NO:29).

Please amend the paragraph on page 12, lines 15-18. A clean version of the amended paragraph follows:

Figure 1H

Comparison of mouse NADE, human HGR74 protein and other homologous rat, mouse and human amino acid sequences. musnade3a (SEQ. ID NO:30); hunade3a1 (SEQ. ID NO:31); hunade3a2 (SEQ.ID NO:32); ratnad3a (SEQ. ID NO:33); ratnad3b (SEQ. ID NO:34); musnade3b (SEQ. ID NO:35); humnade1 (SEQ. ID NO:36); ratnade1 (SEQ.ID NO:37); musnade1 (SEQ. ID NO:38); humnade2 (SEQ. ID NO:39).

Please amend the paragraph on page 14, lines 31-35. A clean version of the amended paragraph follows:

Figure 4A

At residues 88-100, the mouse NADE NES (SEQ. ID NO:40) lies within the C-terminus. A mouse NADE (SEQ. ID NO:41) is aligned with homologous sequences of NADE family members and the NES sequences of HIV Rev (SEQ. ID. NO:42), MAPKK (SEQ. ID NO:43), cZyxin (SEQ. ID. NO:44) and PKI-a (SEQ. ID NO:45).

Please amend the paragraph on page 16, line 36 through page 17, line 22. A clean version of the amended paragraph follows:

This invention provides an isolated nucleic molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described isolated nucleic molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor the isolated nucleic acid is a DNA molecule. In another embodiment of the above described isolated nucleic acid molecule encoding

a polypeptide capable of binding a $p75^{NTR}$ receptor the isolated nucleic acid is a cDNA molecule. In a further embodiment of the above described isolated DNA molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor the isolated nucleic acid is a RNA molecule. In an embodiment of the above described isolated nucleic acid molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, the isolated nucleic acid is operatively linked to a promoter of RNA transcription. In yet another embodiment of the above described nucleic acid molecule, said isolated nucleic acid molecule encodes a neurotrophin associated cell death executor protein. In an embodiment of the above described nucleic acid molecule, said isolated nucleic acid molecule comprises a sequence of AATTG TCTAC GCATC CTTAT GGGGG AGCTG TCTAA C (SEQ.ID NO:1).

Please amend the paragraph on page 19, line 17 through page 20, line 11. A clean version of the amended paragraph follows:

This invention provides a vector which comprises the isolated nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, operatively linked to a promoter of RNA transcription. In an embodiment of the invention, where in the vector which comprises the isolated nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, operatively linked to a promoter of RNA transcription is a plasmid. In another embodiment the above described isolated nucleic acid molecule which is a cDNA molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, encodes a human or mouse protein. In yet another embodiment the above described isolated nucleic acid molecule is a cDNA molecule wherein the nucleic acid molecule encodes a polypeptide capable of binding a $p75^{NTR}$ receptor comprising the amino acid sequence set forth in Figure 1A (SEQ ID NO:13). In a further embodiment the above described isolated

nucleic acid molecule is a cDNA molecule wherein the nucleic acid molecule encodes a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described isolated nucleic acid molecule which is a cDNA molecule wherein the nucleic acid molecule encodes a polypeptide capable of binding a $p75^{NTR}$ receptor which is a mouse, rat or human protein. In yet another embodiment of the above described isolated nucleic acid molecule which is a cDNA molecule, said isolated nucleic acid molecule comprises the nucleic acid sequence set forth in Figure 1G-1 (SEQ ID NO:29).

Please amend the paragraph on page 25, lines 5-32. A clean version of the amended paragraph follows:

This invention provides a purified a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described purified polypeptide capable of binding $p75^{NTR}$ receptor is encoded by the isolated nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment the above described polypeptide capable of binding a $p75^{NTR}$ receptor is a fragment of the purified polypeptide capable of binding a $p75^{NTR}$ receptor. In another embodiment the above described purified polypeptide capable of binding a $p75^{NTR}$ receptor has substantially the same amino acid sequence as set forth in Figure 1A (SEQ ID NO:13). In a further embodiment the above described purified polypeptide capable of binding a $p75^{NTR}$ receptor having an amino acid sequence as set forth in Figure 1A (SEQ ID NO:13). In yet another embodiment the above described polypeptide capable of binding a $p75^{NTR}$ receptor has an amino acid sequence as set forth in Figure 1A (SEQ ID NO:13). In a further embodiment, the above described polypeptide capable of binding a $p75^{NTR}$ receptor is a vertebrate polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described polypeptide capable of

binding a $p75^{NTR}$ receptor comprises a neurotrophin associated cell death executor protein. In yet another embodiment of the above described polypeptide capable of binding a $p75^{NTR}$ receptor comprises NCLRILMGELSN (SEQ. ID NO:2).

Please amend the paragraph on page 26, line 1-9. A clean version of the amended paragraph follows:

As used herein, a polypeptide capable of binding a $p75^{NTR}$ receptor having "substantially the same" amino acid sequences as set forth in Figure 1A (SEQ ID NO:13) is encoded by a nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, said nucleic acid having 100% identity in the homeodomain regions, that is those regions coding the protein, and said nucleic acid may vary in the nucleotides in the non-coding regions.

Please amend the paragraph on page 26, line 29 through page 20, line 1. A clean version of the amended paragraph follows:

This invention provides a polyclonal antibody directed to an epitope of the purified protein having the amino sequence as set forth in Figure 1A (SEQ ID NO:13). In a further embodiment the above described monoclonal or polyclonal antibodies are directed to the polypeptide capable of binding a $p75^{NTR}$ receptor, having the amino sequence as set forth in Figure 1A(SEQ ID NO:13).

Please amend the paragraph on page 59, line 35 through page 60, line 34. A clean version of the amended paragraph follows:

DNA construction.

A full length mouse NADE cDNA was constructed on pBluescript II vector by the ligation of the partial NADE cDNA (7-524) and 5'-RACE product. PCR cloning techniques were used to replace the

stop codon and add the 5' *Xho*I site and 3' *Bam*HI site of a full length NADE cDNA. pcDNA3.1(-)Myc-HisA/NADE was constructed by insertion of a full length NADE cDNA to *Xho*I-*Bam*HI site of pcDNA3.1(-)Myc-HisA (Invitrogen). Human NADE cDNA was amplified using a Jurkat T cell cDNA library and cloned to pcDNA3.1(-)Myc-HisA pcDNA3/rat $p75^{NTR}$ was constructed by insertion of a full length rat $p75^{NTR}$ cDNA to *Eco*RI site of pcDNA3(Invitrogen). pGEX4T-1/rat $p75^{NTR}$ICD was constructed by insertion of amplified rat $p75^{NTR}$ICD(a. a. 338-396) to pGEX4T-1(Pharmacia). Mutant NADE expression plasmids, pcDNA3.1(-)Myc-HisA/muNADE (Cys102Ser) and pcDNA3.1(-)Myc-HisA/muNADE(Cys121Ser), were constructed by PCR-based site-direct mutagenesis methods (29). pELAM-Lu for luciferase reporter assay was constructed by insertion of NF-κB binding site of E-selectin promoter region (-730 - 52) to pGL3-Basic *Sac*I-*Bgl*II site. Expression plasmids of GFP-fused NADE proteins were made following: The cDNA of GFP was cloned into NheI-XhoI-cut pcDNA3.1-mouse NADE as a PCR product amplified with the primers 5"-CTAGCTAGCATCATGGTGAGCAAGGGCGAG-3" (SEQ. ID NO:3) and 5"-CCGCTCGAGTCTTGTACAGCTCGTCCAT-3" (SEQ. ID NO:4) using pEGFP-N2 (Clontech) as a template. The deletion mutants delta 101-124-GFP and delta 91-124-GFP were constructed by inserting an XhoI-BamHI-cut PCR fragment generated with Expand high fidelity Taq polimerase (Boehringer Mannheim) into XhoI-BamHI-cut pcDNA3.1-GFP using the primers
5"-ATCCTCGAGCGATCATGGCCAATGTCCAC-3" (sense)(SEQ. ID NO:5),
5"-ATCGGATCCTCTCAGCTGTAGCTCCCT-3" (antisense) (SEQ. ID NO:6) and
5"-ATCGGATCCGATCTCTCTCATCTCCTC-3" (antisense)(SEQ. ID NO:7).

Please amend the paragraph on page 60, line 36 through page 61, line 6. A clean version of the amended paragraph follows:

The mutagenic primers
(5'-AAAGCTTAGGGAGGCACAGCTGAGAAA-3" (SEQ. ID NO:8),
5"-TTTCTCAGCTGTGCCTCCCTAAGCTTT-3" (SEQ. ID NO:9),

5"-ATCCGGAGAAAGGCTAGGGAGGCACA-3" (SEQ. ID NO:10), and 5"-TGTGCCTCCCTAGCCTTTCTCCGGAT-3") (SEQ. ID NO:11) were used to obtain L97A-GFP and L94, 97A-GFP in which Leu94 and Leu97 are replaced with Ala. In all constructs, mutations were verified by sequencing.

REMARKS

The specification has been amended to include reference to SEQ ID NOs. An annotated version of the amended paragraphs of the specification showing all changes relative to the previous version of that paragraph is attached hereto as **Exhibit D.**

The June 1, 2001 Notice states that the subject application does not comply with the Sequence Rules and that applicants must provide 1) a computer readable form (CRF) copy of the "Sequence Listing"; 2) an initial paper copy of the "Sequence Listing", and 3) a statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. §1.821(e) or §1.821(f) or §1.821(g) or §1.825(b) or §1.825(d).

In response, applicant submits herewith a substitute computer readable form (CRF) of the "Sequence listing" in ASCII (DOS) format on the enclosed computer diskette.

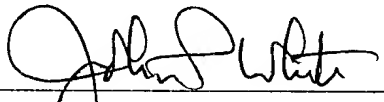
Applicants further submit a paper copy of the Sequence Listing, attached herewith as **Exhibit B**, and a Statement of Compliance Under 37 C.F.R. §1.821(f) attached hereto as **Exhibit C**, certifying that the computer readable form as required by 37 C.F.R. §1.821(e) is identical to the paper copy of the Sequence Listing attached as **Exhibit B**. Applicants believe that the enclosed substitute C.R.F., the paper copy of the Sequence

Listing (**Exhibit B**) and Statement of Compliance Under 37 C.F.R. §1.821(f)) (**Exhibit C**) now fully complies with the requirements of §1.821 through 1.825.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorney invites the Examiner to telephone at the number provided below.

No fee is deemed necessary in connection with the filing of this Amendment. However, if any other fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,



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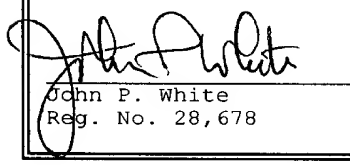

6/15/01

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